

## POPULATION GENETICS OF MARINE SPECIES OF THE PHYLUM ECTOPROCTA

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The literature of marine invertebrate zoology contains relatively few references to genetic studies. Buzzati-Traverso, in a summary of accomplishments and a prospective future of marine biology, declared that knowledge of the genetics of marine organisms was in its infancy and that an understanding of evolutionary processes in the ocean required much more knowledge in that area (Buzzati-Traverso, 1960). Only a few investigations have detected unit genes in the Mendelian sense in which alleles with contrasting phenotypic expression were shown to segregate in the progeny. A partial list of works of this type includes those on the amphipod *Gammarus chevreuxi* (Sexton and Clark, 1936), the copepod *Tisbe reticulata* (Bocquet, 1951; Battaglia, 1958), the isopod *Jaera marina* (Bocquet, 1953), the polychaete *Pomotoceros triqueter* (Føyn and Gjølén, 1954), and the ascidian *Botryllus schlosseri* (Sabaddin, 1959; Milkman, 1967). Most investigations began by noting the occurrence of color polymorphism in the species concerned and led to the elucidation of a simple genetic controlling mechanism. A few other aspects of marine genetics have received brief attention. The cytogenetics of the gastropod *Thais lapillus* in different environments was studied (Staiger, 1954), and the polygenic basis of sex ratios in the copepod *Tigriopus* was investigated (Ar-Rushdi, 1958). Barigozzi (1960) speculated on the nature of genetic systems in sessile marine organisms. Useful reviews of the state of marine genetics have been published (Buzzati-Traverso, 1960; Ray, 1960; Battaglia, 1965). The chief conclusion to be gained is that genetic studies on marine animals have been extremely limited in scope and theoretical significance.

Other than chromosome counts of a few species (Makino, 1951), nothing is known of the genetics of the Phylum Ectoprocta. Marine ectoprocts are abundant, sessile benthic animals that feed on phytoplankton by means of a ciliated crown of tentacles, the lophophore. The initial phase is a solitary planktonic larva, which soon settles. A colony is formed by successive asexual budding of new individuals from previously formed individuals. Thus each individual (zooid) of an ectoproct colony is genetically identical.

Several fundamental aspects of the life cycle of species of ectoprocts are poorly known. A point of special confusion has involved the mode of fertilization because the majority of ectoprocts are hermaphroditic. According to Hyman (1959), most specialists believe that hermaphroditic ectoprocts are self-fertilizing. Silén (1966) and Bullivant (1967) concluded from observations of sperm release into sea water that at least some species may practice outbreeding. Resolution of the question of either autogamy or intra-colony selfing *versus* outbreeding, and the understanding of genetic systems in general have been impeded by two practical

difficulties. (1) Morphological variants of potential genetic origin have not been recognized in any ectoproct species; and (2) ectoprocts have not been successfully crossed in the laboratory. One or both of these difficulties apply equally well to many marine animals.

For the last decade, and especially since 1966 when the papers of Hubby and Lewontin, and Lewontin and Hubby appeared, electrophoretic and histochemical-staining techniques have been utilized for the detection of genetic variants at single loci. Crude protein extracts are prepared from individual organisms and spotted onto starch or polyacrylamide gels. Polypeptides coded by each allele of a locus migrate a characteristic distance in an electric field applied across the gel. The mobility of the polypeptide depends on its electrical charge, size and conformation. Mutations that alter these properties of a polypeptide will alter its mobility; therefore the number and kinds of alleles at a locus can often be determined by the electrophoretic pattern displayed by the corresponding polypeptides after fixation and staining by the proper histochemical means.

A considerable body of literature on electrophoretic variants of enzymes now exists and much of it concerns genetic variation (reviewed by Shaw, 1965). The use of electrophoresis in population genetics has been strikingly exploited in studies of *Drosophila* (for example, Hubby and Lewontin, 1966; Lewontin and Hubby, 1966; Johnson, Kanapi, Richardson, Wheeler and Stone, 1966; Yarbrough and Kojima, 1967; Kojima and Yarbrough, 1967; Stone, Wheeler, Johnson and Kojima, 1968; O'Brien and MacIntyre, 1969). A beginning has been made in the application of these techniques to other organisms, most notably by Manwell in taxonomic problems of marine invertebrates (Manwell, 1966; Manwell and Baker, 1963, 1968; Manwell, Baker, Ashton and Corner, 1967). Much of the protein variation in his research was ascribed to genetic causes, but gene loci were not usually identified.

We used electrophoretic techniques to identify gene loci in local populations of several species of marine ectoprocta. Results for the two species best known are reported here. Where our collections were sufficient, gene and genotype frequencies were determined for individual loci, and the amount of genetic variation was calculated.

*Bugula stolonifera* is erect and foliaceous in growth form, and is lightly calcified, whereas *Schizoporella unicornis* grows as an encrusting, highly calcified sheet (Schopf and Manheim, 1967, 1968). Each of these species is widely reported from the Atlantic Coast of North America, England, and Western and Southern Europe (Ryland, 1960, 1965; Maturo, 1966).

#### MATERIALS AND METHODS

Ectoproct colonies were collected in the vicinity of Woods Hole, Massachusetts. *Schizoporella unicornis* was obtained from pilings of the Marine Biological Laboratory (MBL) dock facing into Vineyard Sound, from a floating dock in Green Pond, Massachusetts, 10 km east of Woods Hole, and from a dredge haul from Vineyard Sound about 35 km southwest of Woods Hole (see Fig. 6). Most of the *Bugula stolonifera* came from metal drums used as floats for the MBL supply department area in Eel Pond, Woods Hole. Supplementary collections were taken from Green Pond.

Colonies were kept alive in running seawater tanks. They were prepared for electrophoresis by first removing any intimately associated fauna, such as amphipods and nematodes from *Bugula*, and overgrown barnacles, tunicates and bivalves from *Schizoporella*. Portions of *Bugula* colonies containing 1000–1500 living zooids were triturated in a tissue grinder in cold 12½% sucrose buffer. About 500 zooids per colony of the much more heavily calcified *Schizoporella* were ground in sucrose-buffer in an Albers micromortar (Thomas Co.). Ground samples were centrifuged at room temperature for 15 minutes at 3500 rpm in

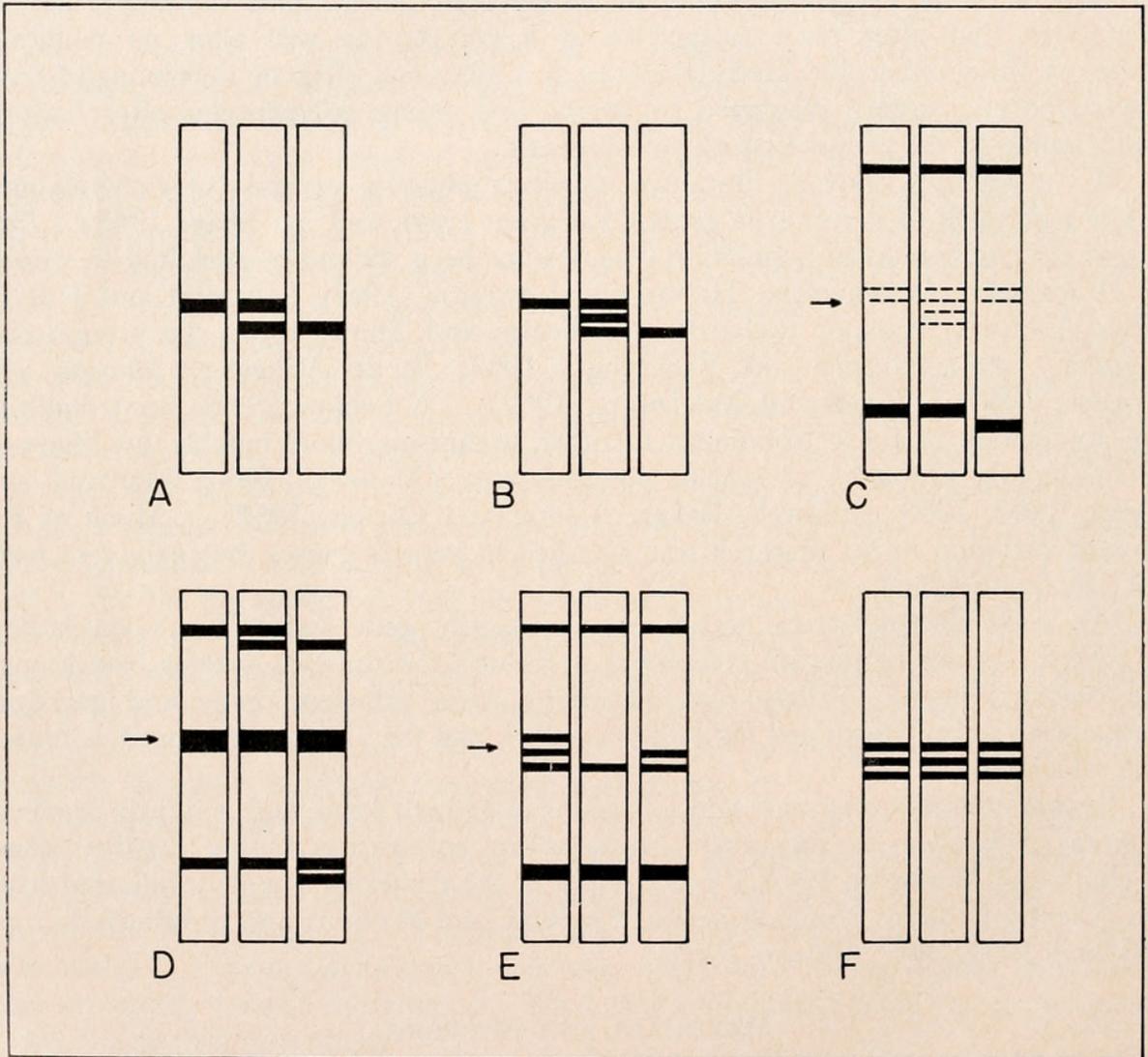


FIGURE 1. Diagrammatic sketch showing patterns of bands illustrating genetic loci: A. Simple diallelic system with an upper band homozygote, a heterozygote, and a lower band homozygote; B. Diallelic system with an upper band homozygote, a heterozygote with a hybrid band, and a lower band homozygote; C. Inhibition of middle zone without affecting upper or lower zones; D. Monomorphic middle zone which does not vary in position relative to zones of defined loci above and below it; E. Polymorphic middle zone which varies in position relative to zones of defined loci above and below it; F. Zone of several bands of possibly more than one locus but which cannot be further resolved by techniques at hand. Arrows denote the zone under consideration.

1 ml polyethylene centrifuge tubes and 10  $\mu$ l of supernatant were pipetted onto the gels.

Electrophoresis was performed with a vertical electrophoresis cell (E-C Corporation, Philadelphia, Pennsylvania) similar to the method of Hubby (1963) and Hubby and Lewontin (1966). However, a 6½% polyacrylamide gel was used throughout because of its superior resolution of bands of malate dehydrogenase. Electrophoresis was conducted for 3 hours at 350–400 V, and 50–100 mA for most analyses. Recirculating water from an ice bath kept the buffer temperature near 15° C. Usually 23 colonies were electrophoresed simultaneously. The 24th slot on the gel was reserved for Bovine serum albumin (Nutritional Biochemicals Corp.). The major, fast moving band of Bovine albumin served as a reference standard for distance in all analyses.

Electrophoresis of nonspecific esterases was conducted using 0.2 M Tris-glycine buffer, pH 8.3. Gels were preincubated in 150 ml of 0.5 M boric acid at 5° C for one hour and then transferred to 150 ml of 0.1 M phosphate buffer solution, pH 6.5 at room temperature, containing 40 mg of  $\alpha$ -naphthyl acetate as substrate and 75 mg of Fast Red TR Salt as dye coupler. Staining was complete in two hours, after which gels were photographed and described, and then preserved in 4% acetic acid.

Esterase inhibition studies were conducted on 3–5 colonies with eserine sulfate, EDTA, and phenylmethylsulfonyl fluoride (PMSF). In alternate substrate studies, 40 mg  $\beta$ -naphthyl acetate and 0.3 ml  $\alpha$ -naphthyl butyrate were substituted for the usual substrate.

Determinations of malate dehydrogenase were made using 0.2 M Tris-glycine buffer, pH 8.3. Tris-borate buffer, pH 8.9, and Tris-EDTA-borate buffer, pH 8.9, were also tried but did not yield good resolution. After electrophoresis, gels were incubated in 150 ml of 0.005 M Tris-HCl-buffer, pH 8.5 at room temperature, containing 20 mg of NAD, 60 mg of Nitro BT chloride and 3 mg of phenazine methosulfate. Bands were developed after 1½–2½ hours of incubation in the dark.

Electrophoretic determinations of "leucine" aminopeptidase were made with 0.1 M Tris-borate buffer, pH 8.9, containing 1mM MgCl<sub>2</sub>. Following a preincubation period of 30 minutes in 125 ml 0.1 M Tris-maleate buffer, pH 5.3, and 40 mg of Leucyl- $\beta$ -naphthylamide at room temperature, 75 mg of Fast Black K salt (Pfaltz and Bauer, Co., New York) were added. Bands developed during the following 1–2 hours. Occasionally it was necessary to remove the old staining solution and add fresh substrate and dye coupler to permit development of faint bands.

#### IDENTIFICATION OF GENETIC LOCI

In order to obtain genetic information from electrophoresis gels, a means must be found to estimate the number of gene loci and the number of alleles per locus from the pattern of enzymatically active bands. Much of the difficulty of interpretation is obviated if organisms suspected of carrying different allelic combinations at a locus are crossed under controlled laboratory conditions. The progeny are then scored for patterns and these are compared to expected genotypes. Most discoveries of electrophoretic variants under genetic control have been made in maize, *Drosophila*, *Mus*, and other organisms that are readily crossed (Shaw, 1965). Many marine invertebrates, and ectoprocts in particular, have never been success-

fully crossed in the laboratory. Therefore three criteria that do not involve crosses were utilized in this study in estimating the number of genetic loci (Fig. 1).

(1) A codominant diallelic or triallelic locus in which each allele produces a single enzyme band of characteristic mobility is easily recognized (Fig. 1A). Heterozygotes have the bands of both alleles. Each heterozygote band is usually lighter than the corresponding band of the homozygotes since it is produced by a single dose of the gene. This type of band pattern has been noted repeatedly in reference in *Drosophila* and other organisms (see, for example, Wright, 1963; Beckman and Johnson, 1964a; 1964b; Scandalios, 1969). It also occurs in the ectoproct species under study, particularly in esterase gels of *Bugula stolonifera* and LAP gels of both species. We regard such a pattern as a reliable criterion for the existence of a polymorphic genetic locus.

In multimeric enzymes, polypeptide subunits may form one or more intermediate bands approximately evenly spaced between the bands of homozygote mobilities. Commonly a single intermediate band is found in heterozygotes (Fig. 1B) which presents no problem in interpretation. However, the genetic relationships of multimeric enzymes manufactured by several mobility alleles can be complicated (for example, see Burns and Johnson, 1967). Complex loci of this type occur in the species *Bugula turrita* and *B. simplex* (unpublished data) but not in *B. stolonifera* and *S. unicornis*.

(2) The use of specific inhibitors and alternate substrates also permits the recognition of loci. The rationale is that a band or a band complex differentially affected by these agents in comparison to adjacent zones probably includes polypeptides that originate from a single locus (Fig. 1C). This conclusion does not extend to groups of bands with widely different mobilities that happen to respond to the same inhibitors or alternate substrates.

Gels stained for nonspecific esterases with  $\alpha$ -naphthyl acetate usually show numerous zones of activity that represent several enzymes. Alternate substrates and various inhibitors with partial specificity have been used to distinguish classes of esterases (Allen, 1961; Ogita and Kasai, 1965; and Manwell *et al.*, 1967). Eserine sulfate inhibits certain cholinesterases, and EDTA inhibits some aryl-esterases (Augustinsson, 1961). PMSF inhibits acetylcholinesterase, trypsin and chymotrypsin (Gold, 1967). The alternate substrates  $\beta$ -naphthyl acetate and  $\alpha$ -naphthyl butyrate are preferentially acted upon by certain esterases.

(3) Bands are not randomly scattered over the gels, but are usually localized into discrete zones. A zone is considered representative of a single locus if variation within it appears independent of other zones of the gel (Fig. 1D). Therefore, a zone of activity appearing as a band of uniform mobility is regarded as monomorphic if it is adjacent to zones definitely identified as polymorphic (Hubby and Lewontin, 1966). Similarly, zones containing bands of variable mobility but difficult to interpret genetically are considered polymorphic if they are adjacent to zones identified as either monomorphic or polymorphic by other criteria (Fig. 1E).

We have no criterion for distinguishing loci where series of closely adjacent bands of uniform mobility are present other than the use of inhibitors and alternate substrates as in the case of esterases (Fig. 1F). A series of bands may represent several independent monomorphic loci, a sharing of subunits in a multimeric system or an artifact of conditions of the analysis. In this study, series of adjacent

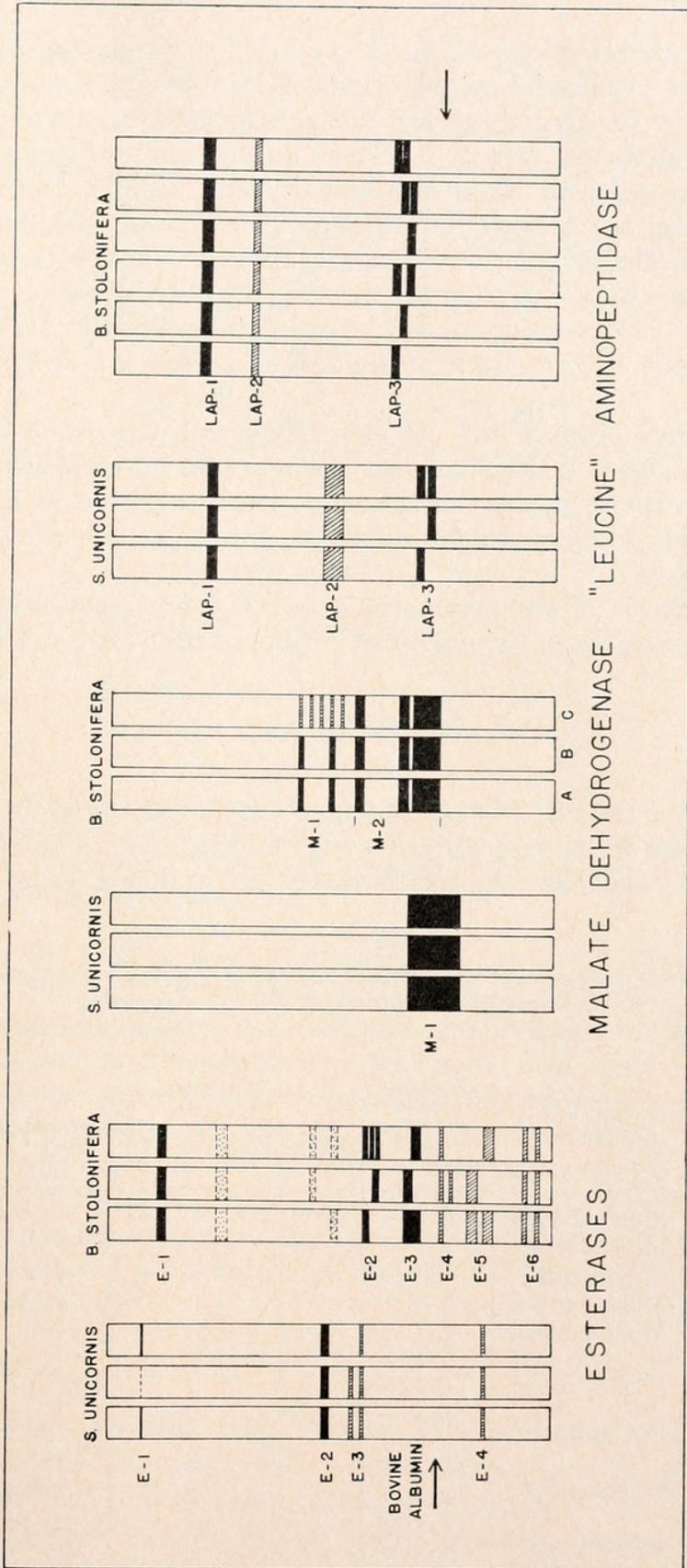


FIGURE 2. Diagram illustrating patterns of banding of *Schizoporella unicornis* and *Bugula stolonifera* in esterases ( $n = 48$ ;  $n = 44$ , respectively), malate dehydrogenase ( $n = 26$ ;  $n = 54$ ), and "leucine" aminopeptidase ( $n = 126$ ;  $n = 43$ ). Dark bands represent heavily stained zones of enzyme activity, hatched bands stand for more lightly stained zones, and stippled bands faint zones.

bands of uniform mobility have been conservatively regarded as belonging to the same locus.

Based on these determinations we have employed a terminology for enzymes and genetic loci based principally on the system of Hubby and Lewontin (1966). For example, in *B. stolonifera* there are 6 zones of esterase activity under the control of separate genetic loci (Fig. 2). These enzymes are designated esterase-1 through esterase-6 in order of increasing mobility and distance from the origin. The corresponding loci are termed E-1 through E-6. Thus, the fourth zone of esterase activity from the origin is the enzyme esterase-4 and it is controlled by the locus E-4. The esterase-4 enzyme has two mobility variants that migrate distances of 1.01 and 1.04 relative to that of the bovine albumin standard. The mobility variants belong to two alleles of the E-4 locus, and are designated E-4<sup>1.01</sup> and E-4<sup>1.04</sup> (Table I).

Alleles of some loci produce two or more enzyme bands when homozygous. The mobility of such alleles is designated by a single superscript which is the midpoint between the outermost bands. Thus, the monomorphic locus M-2<sup>0.85</sup> of *Bugula stolonifera* corresponds to three bands of malate dehydrogenase ranging between 0.75 and 0.95 in mobility.

The number of alleles of the polymorphic loci E-3 in *Schizoporella unicornis* and M-1 in *B. stolonifera* is as yet unknown. Superscripts have not been applied to these loci.

## RESULTS

### Esterases

*Schizoporella unicornis* and *Bugula stolonifera* are quite unlike in the number and mobilities of zones of esterase activity (Fig. 2, Table I). The simplest band pattern occurs in *S. unicornis*, in which there are 4 major zones of activity,

TABLE I  
Locus and allele nomenclature for esterases and biochemical characterization of loci in *Schizoporella unicornis* and *Bugula stolonifera*

Species	Locus Monomorphic or Polymorphic	Nomenclature of locus and alleles	Inhibitor			Substrate utilization		
			Eserine	PMSF	EDTA	$\alpha$ -naphthyl acetate	$\beta$ -naphthyl acetate	$\alpha$ -naphthyl butyrate
<i>Schizoporella unicornis</i>	Monomorphic	E-1 <sup>10</sup>	++	-	-	+	-	-
	Monomorphic	E-2 <sup>66</sup>	-	-	-	++	+	++
	Polymorphic	E-3	-	-	-	++	+	+
	Monomorphic	E-4 <sup>1.14</sup>	-	-	-	++	+	+
<i>Bugula stolonifera</i>	Monomorphic	E-1 <sup>16</sup>	++	-	-	++	+	-
	Polymorphic	{ E-2 <sup>78</sup>	-	-	-	+	+	+
		{ E-2 <sup>81</sup>						
	Polymorphic	{ E-3 <sup>90</sup>	-	++	-	+	+	+
		{ E-3 <sup>94</sup>						
	Polymorphic	{ E-4 <sup>1.01</sup>	+	-	-	+++	++	+
		{ E-4 <sup>1.04</sup>						
Polymorphic	{ E-5 <sup>1.11</sup>	+	++	-	++	+	+	
	{ E-5 <sup>1.16</sup>							
Monomorphic	E-6 <sup>1.29</sup>	-	-	-	+	-	-	

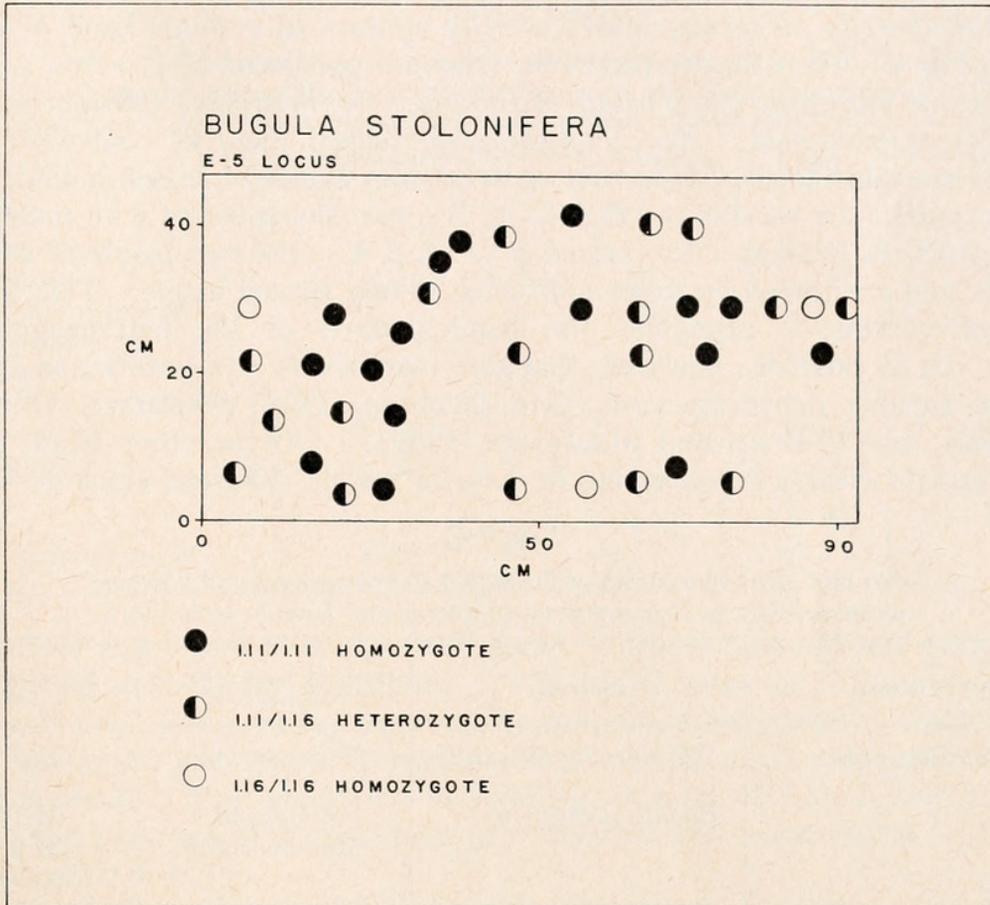


FIGURE 3. Plot of areal distribution of genotypes of *Bugula stolonifera* (locus E-5) on a floating drum at Eel Pond. This pattern of random distribution of a genotype is typical for other loci on this drug and on others.

corresponding to 4 gene loci, 3 monomorphic and 1 polymorphic and diallelic (Table I). Esterase-1, the product of the monomorphic E-1 locus, is inhibited by  $1 \times 10^{-4}$  M eserine sulfate, and does not appear when  $\beta$ -naphthyl acetate and  $\alpha$ -naphthyl butyrate are the substrates. Enzymes of the other loci are unaffected by the inhibitors employed and show little differential activity on alternate substrates.

Esterase band patterns are more complex in *B. stolonifera*. The 6 major zones of esterase activity are each controlled by a separate locus, E-1 through E-6. Four of the loci are polymorphic and diallelic (Table I). As in *S. unicornis*, esterase-1 is inhibited by eserine sulfate and does not utilize  $\beta$ -naphthyl acetate and  $\alpha$ -naphthyl butyrate. Esterase 3 is inhibited by PMSF and the two bands of esterase-6 are greatly clarified by EDTA.

#### Malate dehydrogenase

The staining procedure for malate dehydrogenase (MDH) has greater specificity than that for esterases. Nevertheless, several MDH enzymes with different coenzyme and divalent cation requirements are known (Moore and Vilee, 1963; Manwell *et al.*, 1967). In our study, attempts to detect NADP- and  $Mg^{++}$ -dependent MDH were unsuccessful. NAD-dependent MDH, however, was detected in both species.

In *Schizoporella unicornis*, MDH activity appears in a single band of uniform mobility (Fig. 2). It is the product of the monomorphic locus M-1.

The fastest migrating three bands of *Bugula stolonifera* have constant mobilities in all colonies examined. We conservatively regard them as controlled by the same monomorphic locus, M-2. In contrast, the bands of lesser mobility occur as two variants. In variant *A* (Fig. 2, A, B), two sharp bands with mobilities of 0.58 and 0.68 are present. In variant *B* (Fig. 2, C), the two bands of variant *A* are fainter and are joined by three additional, evenly spaced bands. This 5-banded pattern of variant *B* resembles the band pattern of the heterozygote of a tetramer. It is possible, however, that the variation is non-genetic, as has been noted for various dehydrogenases (see Jacobson, 1968; Thornber, Oliver and Scutt, 1968; and O'Brien and MacIntyre, 1969). On the other hand, the two variants remain clearly recognizable in spite of widely different amounts of crude

TABLE II  
*Locus and allele nomenclature for malate dehydrogenase and "leucine" aminopeptidases in Schizoporella unicornis and Bugula stolonifera*

Type of protein	Species	Locus Monomorphic or Polymorphic	Nomenclature of locus and alleles
Malate dehydrogenase	<i>Schizoporella unicornis</i>	Monomorphic	M-1 <sup>.99</sup>
	<i>Bugula stolonifera</i>	Polymorphic Monomorphic	M-1 M-2 <sup>.85</sup>
"Leucine" aminopeptidase	<i>Schizoporella unicornis</i>	Monomorphic	Lap-1 <sup>.31</sup>
		Monomorphic	Lap-2 <sup>.68</sup>
		Polymorphic	Lap-3 <sup>.94</sup> Lap-3 <sup>.98</sup>
	<i>Bugula stolonifera</i>	Monomorphic Monomorphic Polymorphic	Lap-1 <sup>.28</sup> Lap-2 <sup>.43</sup> Lap-3 <sup>.86</sup> Lap-3 <sup>.88</sup> Lap-3 <sup>.90</sup>

protein introduced into the gel slots, different parts of the colony electrophoresed (growing apical zooids, reproducing zooids, and degenerating zooids (brown body regions)), and differences in pH and ionic strength of the buffer. This is good evidence that they have a genetic basis. If the variants are genetic, probable differences in gene frequency exist between Eel Pond and Green Pond. Only 4 of 28 colonies (or 14%) from Eel Pond are variant *B* whereas 11 of 26 (42%) from Green Pond are variant *B*. Variants *A* and *B* of *B. stolonifera* are treated as representative of one polymorphic locus, M-1.

#### "Leucine" aminopeptidase

Zones of activity for "leucine" aminopeptidase (LAP) are similar on gels of *Schizoporella unicornis* and *Bugula stolonifera*. Both species possess three loci controlling LAP, two monomorphic and one polymorphic (Table II). The enzymes of the Lap-1 loci of both species are similar in mobility (Lap-1<sup>.31</sup> in

TABLE III

Amount of polymorphism in esterases, malate dehydrogenase, and "leucine" aminopeptidase for *Schizoporella unicornis* and *Bugula stolonifera*

	<i>Schizoporella unicornis</i>	<i>Bugula stolonifera</i>	Totals	Per cent polymorphism
Esterases				
Monomorphic	3	2	5	
Polymorphic	1	4	5	50.0
Malate dehydrogenase				
Monomorphic	1	1	2	
Polymorphic	0	1	1	33.3
"Leucine" aminopeptidase				
Monomorphic	2	2	4	
Polymorphic	1	1	2	33.3
Totals	6	6	11	42.1
Per cent polymorphism	25.0	54.5	42.1	

*S. unicornis* and Lap-1<sup>28</sup> in *B. stolonifera*). Lap-2 enzymes are quite different in mobilities and staining properties. The Lap-3 locus in *S. unicornis* has two alleles and the corresponding locus of *B. stolonifera* has three. Once again, the protein mobilities are similar (mean mobility of 0.96 in *S. unicornis* and 0.88 in *B. stolonifera*). The similarity in banding pattern of Lap-1 and Lap-3 may indicate the presence of homologous proteins that are conservative to changes in electrophoretic mobility.

Gene and genotype frequencies and polymorphism

Genotype frequencies have been determined for 5 separate polymorphic loci (Table IV). Data for Lap-3 of *Schizoporella unicornis* are given for 3 localities making a total of 7 determinations. Gene frequencies were calculated from genotype frequencies by the formulas:  $p = P + \frac{1}{2}H$ , and  $q = Q + \frac{1}{2}H$ , where p and q

TABLE IV

Summary of gene and genotype frequencies and chi-square agreement with Hardy-Weinberg equilibrium values for *Schizoporella unicornis* and *Bugula stolonifera*

Species	Locality	Sample size	Locus	Gene frequency		Genotype frequency			Chi-Square agreement of genotype frequency with Hardy-Weinberg
				Low mobility allele	High mobility allele	Low mobility homozygotes	Heterozygotes	High mobility homozygotes	
<i>Schizoporella unicornis</i>	MBL Dock	50	Lap-3	0.720	0.280	25	22	3	no significant difference
	Green Pond	43	Lap-3	0.756	0.244	25	15	3	no significant difference
	Vineyard Sound	33	Lap-3	0.485	0.515	9	14	10	no significant difference
<i>Bugula stolonifera</i>	Eel Pond	44	E-2	0.864	0.136	34	8	2	no significant difference
	Eel Pond	43	E-3	0.602	0.398	20	12	11	$P < 0.01$
	Eel Pond	44	E-4	0.955	0.045	40	4	0	no significant difference
	Eel Pond	44	E-5	0.693	0.307	20	21	3	no significant difference

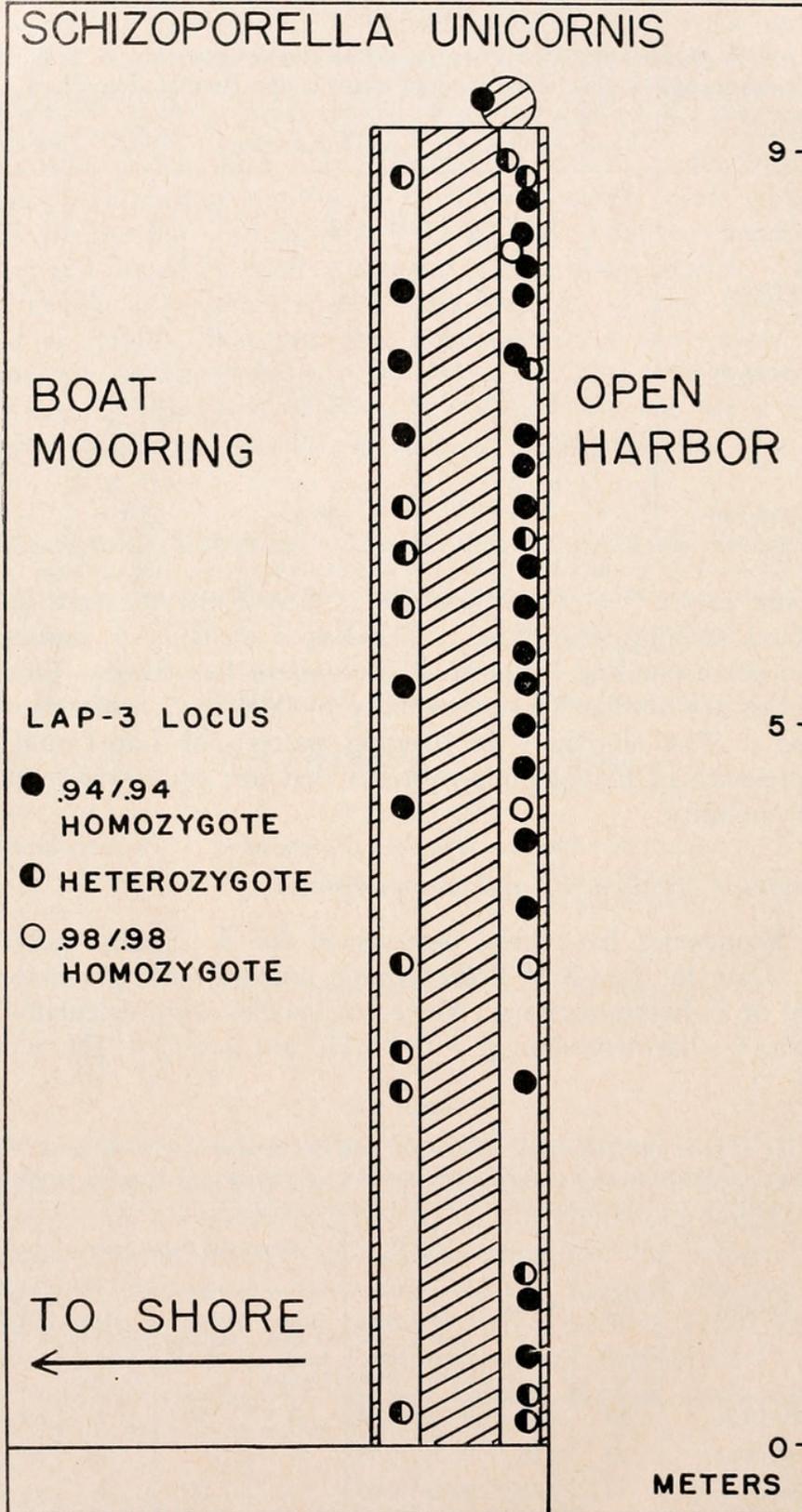


FIGURE 4. Distribution of genotypes of "leucine" aminopeptidase (locus Lap-3) in colonies of *Schizoporella unicornis* occurring on outer floating dock of boat moorings at Green Pond, Massachusetts. White area represents the surface available for colonization. Note that the boat mooring side has a greater frequency of heterozygotes than the open water side. The difference is significant at the 5 per cent level.

are gene frequencies and P, H and Q are the frequencies of homozygote, heterozygote, and other homozygote classes respectively (Falconer, 1960). The Hardy-Weinberg equilibrium distribution of genotypes was calculated from gene frequencies for the 5 loci. Genotypes obtained were compared to expected values for goodness-of-fit (Table IV). Only the genotype frequency of the E-3 locus in *Bugula stolonifera* differs significantly from Hardy-Weinberg equilibrium values. At this locus there is a significant deficiency of heterozygotes ( $P < 0.01$ ).

In *Schizoporella unicornis* 2 of 8 or 25.0% of loci are polymorphic and in *Bugula stolonifera* 6 of 11 or 54.5% of loci are polymorphic. Mean polymorphism is 42.1% (Table III). Combined data from both species show 5 esterase loci (50%), 1 MDH locus (33.3%), and 2 LAP loci (33.3%) to be polymorphic.

#### *Geographic variation in gene and genotype frequencies*

Local populations or demes of *Bugula stolonifera* in Eel Pond extend over areas of at least 3.6 m<sup>2</sup>. Genotypes of the E-5 locus are randomly distributed over this area (Fig. 3).

Collections were made from the floating dock at Green Pond and the pilings at the MBL dock which show the distribution of Lap-3 genotypes of *Schizoporella unicornis* over areas of 11–18 m<sup>2</sup> (Figs. 4, 5). At Green Pond (Fig. 4) there is a possible bias of heterozygotes ( $\chi^2 = 4.26$ ,  $P < 0.05$ ) on the side of the dock which is part of a motor boat mooring. The water on this side is more polluted than that of the free harbor. In contrast, the distribution of genotypes in a sample collected from several pilings of the MBL dock appears to be random (Fig. 5). The depth distribution on the pilings, however, does suggest a non-random distribution of genotypes (Fig. 5). The homozygote Lap-3<sup>94/94</sup> is absent in 22 near surface samples, occurs in 1 of 14 samples of intermediate depth, but accounts for 2 of the 4 deepest samples.

A third geographic comparison is available for populations separated on the order of kilometers (Fig. 6). *S. unicornis* from Vineyard Sound dredgings differs significantly from the MBL dock and Green Pond populations in gene frequency at the Lap-3 locus ( $\chi^2 = 9.3$ ,  $P < 0.01$ , in a comparison of the MBL dock and Vineyard Sound populations). The frequency of Lap-3<sup>98</sup> averages about 24% higher in the Vineyard Sound collection. Samples of MBL dock and Green Pond populations do not show significant differences.

## DISCUSSION

The most important conclusion we can make is that the genetic structure of an ectoproct population appears to be fundamentally like that of genetically well-known terrestrial animals, such as *Drosophila*. This conclusion is, perhaps, surprising, because ectoprocts are colonial, sessile, and for the most part, hermaphroditic. The peculiarities of the ectoproct way of life might be expected to result in unusual sexual and genetic mechanisms. Such mechanisms were not found.

#### *Breeding structure of local populations*

Local populations of *Schizoporella unicornis* at Green Pond and MBL dock, and *Bugula stolonifera* at Eel Pond are composed of several hundred colonies.

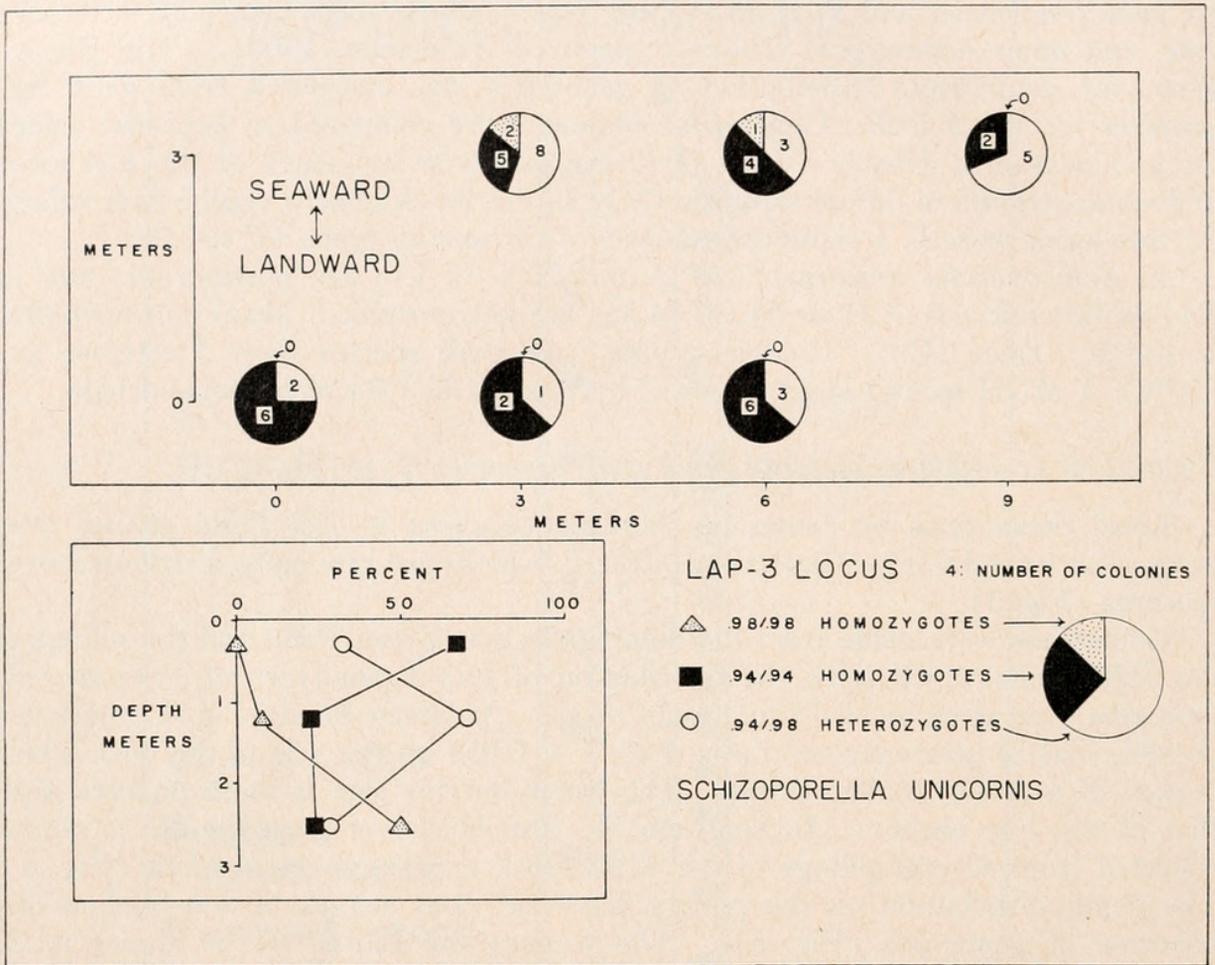


FIGURE 5. Plan view and depth distribution of genotypes of "leucine" aminopeptidase (Locus Lap-3) in colonies of *Schizoporella unicornis* occurring on "islands" of pilings at the MBL dock. In plan view, samples are summed over all depth intervals. Genotypes appear to be randomly distributed, taking into account the small sample size (3-15 colonies) on each set of pilings. In depth, samples are summed over all areas, and by depth interval (0.0-0.6 m, n = 22; 0.6-1.8 m, n = 14; 1.8-3.0 m, n = 4).

Conspicuous aggregations of like genotypes are usually not found, indicating that larvae do not habitually settle in the immediate vicinity of the parental colony. Approximately random distribution of genotypes is the rule. Apparently the sessile mode of adult existence is not a barrier to dispersal of ectoprocts over distances of square meters or tens of square meters.

Genotype frequencies at several loci in these local populations are largely in accord with Hardy-Weinberg equilibrium frequencies. We infer that individuals of each colony freely interbreed with those of other colonies in the local population. Since sperm and eggs are produced by the colonies of these species at the same time, a self-incompatibility mechanism may be present to prevent intra-colony fertilization. Nevertheless, some inbreeding may exist in ectoproct populations, but has escaped detection. As an example, the sample of 50 colonies of *Schizoporella unicornis* from the MBL dock is too small to detect a coefficient of inbreeding (F) of 0.25 at the 0.5 level of significance of the chi-square test.

The close agreement with Hardy-Weinberg genotype frequencies suggests

that alleles at the loci sampled are selectively neutral. However, Lewontin and Cockerham (1958) have shown that statistical agreement with Hardy-Weinberg values can be an insensitive indicator of selection in small population samples. It is distinctly possible that selection pressures of 10–20% are operating against particular genotypes. There is tentative evidence for this viewpoint since genotypes of the Lap-3 locus in *S. unicornis* depart from random distribution along environmental gradients. At Green Pond a positive bias of heterozygotes appears to occur on the side of the floating dock closer to a source of oil and gasoline pollution (Fig. 4). At the MBL dock, the Lap-3<sup>94/94</sup> homozygotes become proportionately more abundant with increasing depth (Fig. 5).

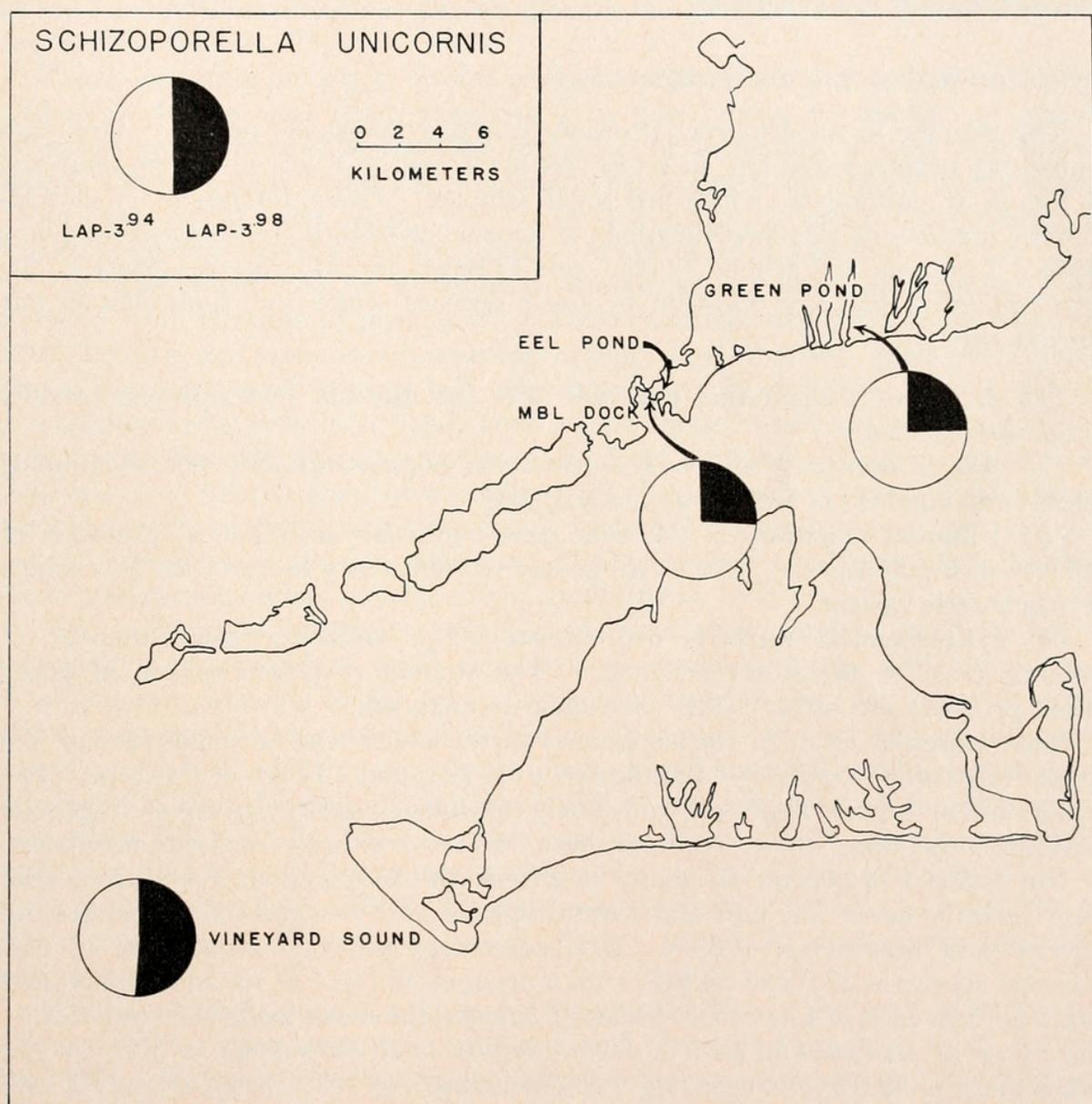


FIGURE 6. Map showing location of collecting localities from Green Pond, MBL dock, and Vineyard Sound dredging, together with gene frequencies for Lap-3 alleles. Note that the coastal samples have nearly identical gene frequencies whereas the Vineyard Sound frequency is distinctly different.

The only locus to show statistically significant departure from Hardy-Weinberg equilibrium values ( $P < 0.01$ ) is the E-3 locus in Eel Pond populations of *B. stolonifera*. There is a marked deficiency of the heterozygote class. Since genotypes at the other sampled loci conform to equilibrium frequencies, self-fertilization or close inbreeding are not responsible. Inbreeding would lead to a deficiency of heterozygotes at all loci. Positive assortive fertilization could account for the deficit of heterozygotes, by which gametes carrying either the E-3<sup>90</sup> or E-3<sup>94</sup> allele preferentially fertilize eggs bearing the like allele. Alternatively, fertilization may be random but heterozygote mortality is greater. Either explanation requires linkage equilibria between E-3 and the other loci sampled. This problem cannot be resolved until more is known of gamete behavior and the relative fitness of E-3 genotypes.

#### *Genetic variability of local populations*

The genome of an ectoproct probably contains thousands of loci. Our data concerning genetic variability in ectoprocts are based on 8 loci in *S. unicornis* and 11 loci in *B. stolonifera*, which are small samples. Other limitations of electrophoretic methods in the determinations of genetic variability are discussed in Shaw (1965), Lewontin and Hubby (1966), and O'Brien and MacIntyre (1969). The most important biases recognized by these authors which are applicable to our research are:

(1) A genetic variant that does not alter the mobility of its protein product will not be detected.

(2) Alleles occurring at low frequencies in populations may not be sampled unless large numbers of individuals are analyzed.

(3) Different mutations of the same gene may cause an identical alteration in mobility of a polypeptide. Thus two genetic variants may be recorded as a single electrophoretic variant.

(4) Histochemical methods are known for a relatively small number of proteins, most of which are enzymes. The amount of polymorphism in genes whose products are other classes of protein is unknown.

Most biases inherent in electrophoresis methodology tend to underestimate the amount of genetic variability. Limitations (1), (2), and (3) are of this type. The limitations in methodology are not likely to cause recent estimates of genetic variability, including those reported here, to be drastically revised downward.

Our analysis of genetic variability in ectoprocts yields estimates consistent with those recently made for man and several species of *Drosophila* (summarized by O'Brien and MacIntyre, 1969). The percentage of polymorphic loci in two ectoproct species is 25.0 and 54.5%, with a mean of 42.1%. If we add unpublished data for 8 loci each of two other species of *Bugula*, the mean value becomes 48.5%. The range of estimates of loci in *Homo sapiens* and *Drosophila* is 30.0–54.0%, with a mean of 42.0%, disregarding a single estimate of 100% based on only 3 loci. The average proportion of the genome heterozygous per individual (calculated after Lewontin and Hubby, 1966) is 0.07 in *S. unicornis* and 0.21 in *B. stolonifera* compared with a range of 0.12–0.33 obtained for man and *Drosophila*.

If these estimates of the amount of polymorphism are typical of the terrestrial

and marine taxa they represent, then little seems to distinguish the colonial, sessile, and hermaphroditic marine ectoprocts from solitary, free living, unisexual terrestrial animals in the genetic variability maintained in populations.

#### *Consequences of spatial separation*

The marine realm is frequently stated to be more homogenous and stable than the terrestrial (David, 1963) with the notable exception of the highly-variable intertidal environment (Knox, 1963). Some scientists have regarded the apparent scarcity of barriers to migration in the sea as evidence of sympatric speciation (Kohn, 1960; Weiser, 1960; and Day, 1963). Ernst Mayr, by no means an advocate of sympatric speciation, considered that free gene flow has led to widespread panmictic conditions in marine animals as opposed to local genetic differentiation (Mayr, 1963).

Two contrasting models of marine ectoproct populations may be proposed. According to the one just cited, extensive gene flow among populations of marine ectoprocts would militate against the formation of semi-isolated local populations. Species would be cosmopolitan aggregations of individuals and major shifts in gene frequencies would only occur over extensive areas differing considerably in important environmental parameters such as temperature and salinity.

The other model calls for a marine environment beset (from an ectoproct point of view) with barriers to gene flow. Micro-environmental changes in substrate relief, texture, and composition, current strength and direction, quality and quantity of nutrients, and salinity and temperature might serve to disrupt genetic communication between local populations. This model would permit divergence in gene frequencies due either to responses to local selection pressures or, in small populations, to genetic drift, or a combination of selection and drift. Small, semi-isolated populations are commonly found in terrestrial animals such as the snail *Cepaea nemoralis* (Cain and Sheppard, 1954), species of *Drosophila* (Wallace, 1968), and certain human populations (Cavalli-Sforza, 1959).

Our evidence is insufficient to fully evaluate these models, but it is more consistent with a model of small, semi-isolated populations than with the concept of widespread panmixia in the sea. An approximate 24 per cent disparity exists in the frequency of alleles of the Lap-3 locus in *S. unicornis* between the Vineyard Sound population and the near-shore Green Pond and MBL dock populations. Whether the allele frequencies change clinally or abruptly through the intervening populations is not yet known. In either case the 35 km interval serves as a partial barrier to gene flow or else stringent differential selection maintains the difference in the face of gene flow. Either the existence of effective barriers to gene flow or strong local selection pressures would disrupt panmixia among populations of marine species.

More work needs to be done to assess the potentialities of dispersal in ectoprocts. The larvae of *S. unicornis* are planktonic and therefore subject to rapid current dispersal. However, the larvae are brooded for several days and are released into the water column for a free life of only hours. They have no digestive tract and are nourished by a yolk supply. Estimates of current pattern and strength (Sumner, Osburn, Cole and Davis, 1913; Coast and Geodetic Survey tidal current charts, 1949) show it requires more than one day for water at the MBL dock to

be transported to the Vineyard Sound location assuming a direct and rapid rate of transport. Thus, biological and physical limitations to rapid dispersal appear to be operative. Such gene flow as does occur between these populations must be via intervening populations.

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#### SUMMARY

Proteins of marine species of the Phylum Ectoprocta can be easily separated by zone electrophoresis on polyacrylamide gels. Many bands revealed by electrophoresis represent the product of individual genetic loci from which gene and genotype frequencies were calculated. Results are reported for 19 loci which are responsible for the formation of esterases, malate dehydrogenase and "leucine" aminopeptidase in *Schizoporella unicornis* and *Bugula stolonifera*.

Observed genotype frequencies correspond closely with those predicted by Hardy-Weinberg equilibria indicating that these marine ectoprocts are dominantly outbreeding and that panmictic local populations cover an area of square meters. In addition, 25.0–54.5% of the diagnosed loci are polymorphic, depending on the species. Forty-two % of all loci identified are polymorphic.

The most important implication of these findings is that the population structure and amount of genetic variability in ectoprocts (and by implication in other marine organisms) is fundamentally similar to that of terrestrial organisms. If these tentative conclusions are substantiated by subsequent work, the processes of speciation in marine organisms may differ little from those proposed in the better documented research on terrestrial animals.

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